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Rheopheresis in Patients with Ischemic Diabetic Foot Syndrome: Results of an Open Label Prospective Pilot Trial

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Abstract: Rheopheresis is a specific application of membrane differential filtration, synonymous with double filtration plasmapheresis, for extracorporeal hemorheotherapy. Safety and efficacy of Rheopheresis for wound healing and skin oxygenation were investigated in patients with ischemic diabetic foot syndrome. Eight patients with type 2 diabetes mellitus and non-healing foot ulcers caused by severe ischemic diabetic foot syndrome were treated by a series of seven Rheopheresis sessions in a time span of 11 weeks. Wound healing had not been detectable under conditions of standardized wound care during at least 2 months. Wound status was classified by its morphology, severity and location, according to the criteria of Wagner. Transcutaneous oxygen pressure ($tcPO_2$), laser Doppler flowmetry and vital capillary microscopy were repeatedly performed to monitor the effects of the Rheopheresis treatment series on microcirculation and skin blood flow. Laboratory parameters of blood rheology, endothelial function and inflammatory state were measured in addition to safety parameters. In four patients (baseline Wagner stage 2), Rheopheresis accelerated wound healing of foot ulcers and was associated with an improvement of Wagner stage and a pronounced increase in $tcPO_2$. In two patients (baseline Wagner stage 2), wound healing was unchanged but mean $tcPO_2$ increased, allowing successful minor amputation. Values of $tcPO_2$ remained stable and enhanced for the 3 months follow-up period. In two patients (baseline Wagner stage 4 or 5), no improvements in foot lesions were observed within the treatment period. As an adjunct therapeutic option, Rheopheresis may preserve a functional lower extremity, delay amputation or reduce the extent of amputation. **Key Words:** α_2-macroglobulin—Fibrinogen—Ischemic diabetic foot syndrome—LDL cholesterol—Microcirculation—Rheopheresis—Transcutaneous oxygen tension—Wound healing.

Diabetic foot syndrome is a frequent complication of long-standing type 2 diabetes mellitus, often leading to lower-extremity amputations. This constitutes a significant risk factor for additional amputations and increased mortality (1). Peripheral arterial disease is 2.5–6 times more frequent in diabetic than in non-diabetic patients, and tends to occur 10 years earlier. The overall cumulative lifetime frequency of amputations in type 1 and type 2 diabetic patients is approximately 15% in both Europe and in the United States (2). In the US, more than 51 000 amputations of the lower extremities are performed annually due to diabetic foot syndrome, 10% of these being major amputations (3). The chances of full rehabilitation after amputation are poor in diabetic patients compared with non-diabetic amputees. Revascularization procedures are also less successful in diabetic patients than in non-diabetic cohorts (4).

The multifactorial pathogenesis of ischemic diabetic foot ulceration is based on multiple interactions which involve both micro- and macroangiopathy. These interactions are associated with blood flow alteration, reduced tissue oxygenation, endothelial dysfunction and infection. Both neuropathy and angiopathy lead to functional disturbances in macrocirculation and skin microcirculation. An essential factor in infection control, wound healing and tissue regeneration is adequate blood supply of tissue oxygen, nutrients and soluble mediators. Foot ulcers in patients with diabetes usually have mixed ischemic and neuropathic components. Lack of perfusion negatively affects wound healing and can result in rapid tissue death. Specific diabetic microangiopathy, a

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functional disturbance without visible morphological changes or occlusive capillary disease, adds to the macrovascular disease. The complete network of pathogenic mechanisms that lead to the development of severe tissue ischemia in the diabetic foot and which are initiated by microenvironmental changes in small blood vessels with loss of endothelial function, is not completely understood (5,6).

When the average transcutaneous partial oxygen pressure ($tcPO_2$) levels fall to under 10 mm Hg, the prognosis for non-healing ulcers on the feet of diabetic patients becomes distinctly unfavorable, and generally ends in amputation (7). A decrease in $tcPO_2$ is the first sign of abnormal oxygen transport to the tissues, and preceeds the reporting of first clinical symptoms by the patient (8). Therefore, $tcPO_2$ measurements are widely used as a diagnostic tool for the assessment of tissue perfusion, trophic disorders and severity of foot ischemia. Increases in $tcPO_2$ levels can be used to select appropriate treatment options and can be employed as relevant criteria for delaying amputation or for performing minor instead of major amputation (9,10).

Rheopheresis is a specific application of membrane differential filtration (MDF), synonymous with double filtration plasmapheresis for extracorporeal hemorheotherapy (Fig. 1) (11). An exactly defined spectrum of high molecular weight proteins (such as LDL cholesterol, fibrinogen, α_2-macroglobulin, von Willebrand factor (vWF) and fibronectin) is eliminated from human plasma, resulting in a pulse of lowered blood and plasma viscosity as well as erythrocyte and thrombocyte aggregation. Hemorheological abnormalities and increased blood viscosity were shown to be associated with elevated levels of these plasma proteins; high levels of these proteins also represent a risk factor for micro- and macrovascular complications which lead to foot ulceration and amputation in diabetic patients (12–15). Repeated pulsed reductions in blood and plasma viscosity by the Rheopheresis treatment series can result in a sustained improvement in microcirculation at a functional level (16). In small blood vessels or capillaries, blood flow is predominantly determined by the viscosity of the plasma; this is due to a phenomenon known as the Fahreus-Lindquist effect, where single blood cells are held within the faster laminar plasma flow in the middle of the microvessel, resulting in a variation in hematocrit of around 10%. Rheopheresis has been successfully investigated in controlled, randomized clinical trials in ophthalmology: it was found to be an effective treatment for selected patients with age-related macular degeneration, a microcirculatory disorder of the retina (16,17). In a pilot trial with patients with diabetic retinopathy, visual function was reported to improve after a series of MDF treatments (18).

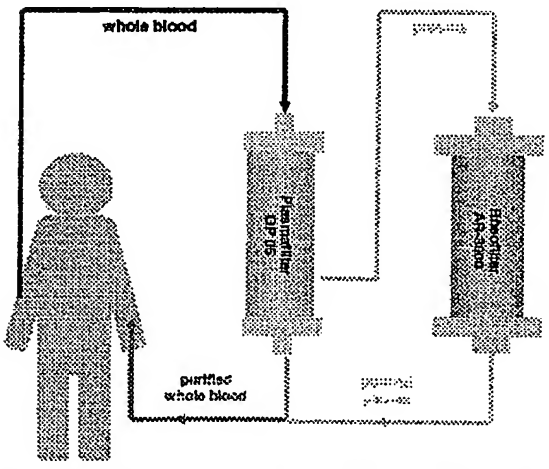

FIG. 1. Schematic drawing of the extracorporeal circuit established for Rheopheresis is shown.

In the open-label, prospective pilot trial presented here, eight patients with non-healing lesions caused by severe ischemic diabetic foot syndrome were treated with Rheopheresis. The primary objective of the study was to evaluate the healing of foot lesions and change of wound status according to the criteria of Wagner (19). Secondary objectives were analyses of changes in $tcPO_2$ at the affected foot and evaluations of laboratory parameters. Furthermore, techniques like laser Doppler flowmetry and computerized videophotometric vital capillary microscopy were both used to determine skin microcirculation and to monitor the process of wound healing. Laser Doppler flowmetry has been used in association with $tcPO_2$ to assess wound healing of ischemic foot ulcerations and amputations in the past (20,21). Skin capillary perfusion, as measured by $tcPO_2$, was described as being more impaired and significantly declined in diabetic patients with a foot risk, reduced arterial circulation and vascular diseases (6). Capillary microscopy was used as a diagnostic tool to indicate capillary blood cell velocity. Patients with diabetic foot syndrome who underwent major amputation had significantly fewer capillaries per mm^2 compared with patients with minor amputations (22).

Microvascular endothelial dysfunction and vascular injury, resulting in part from accelerated atherosclerosis and increased thrombosis, may play crucial roles in the development of diabetic foot syndrome. Circulating cellular adhesion molecules and several acute phase proteins, like fibrinogen or C-reactive

TABLE 1. *Patients with ischemic diabetic foot syndrome—baseline characteristics*

Patient No.	1	2	3	4	5	6	7	8	mean value
Sex	F	M	F	M	M	F	M	M	
Age (years)	72	71	62	72	66	77	73	53	68.3
Weight (kg)	64.2	79.2	62.1	87.7	75.3	101.2	93.1	67	67.0
Diabetes duration (years)	22	23	10	25	11	13	10	11	15.6
Duration of diabetic foot syndrome (months) with optimized glycemic control and under standardized wound care	>2	>2	>2	>2	>2	>2	>2	>2	
Number of treatments	7	7	7	7	7	7	4	7	
Affected foot	right	right	left	right	left	right	right	right	
$tcPO_2$ (mm Hg) at baseline (week 0)	1.1	9.2	9	4.1	3.8	19	1.2	9.1	7.1

protein, are molecular markers of acute or chronic inflammatory response, and have been implicated in the progression of diabetic complications (23,24). To investigate the influence of Rheopheresis on endothelial function at the molecular level, markers associated with vascular damage and inflammatory activity were measured, i.e. soluble intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), E-selectin, thrombomodulin (TM), C-reactive protein (CRP), von Willebrand Factor (vWF), fibronectin and high-sensitivity-CRP (hs-CRP). Especially hs-CRP, a non-specific marker of low-grade systemic inflammation, has been proved to be of predictive value in the assessment of the future development and course of cardiovascular disease (25).

METHODS

Patients

Eight patients (five male, three female) with type 2 diabetes mellitus and refractory, non-healing, chronic foot ulcers caused by severe ischemic diabetic foot syndrome were investigated. Mean patient age was 68.3 years (range 53–77 years). Mean duration of type 2 diabetes mellitus was 15.6 years (range 10–25 years). The baseline value of $tcPO_2$ at the affected foot was lower than 20 mmHg in all patients included in the study. Complete baseline patient characteristics, laboratory and safety parameters are listed in Tables 1,2. All patients had at least one ischemic foot with non-healing lesions. Patients were monitored by a specialized foot care team. The ulcer area was documented at each visit and the metabolic control was optimized. Individualized topical antibiotic treatment and sterile dressings were used depending on the site and character of the ulcer. The ulcers were surgically debrided of necrotic tissue and callus when considered necessary. Off-loaded protective shoewear with individually fitted insoles were used, and external pressure against the ulcer was frequently inspected at each visit and corrected when required. These measures of standardized wound care preceded the investigation and were continued throughout the entire study period, but wound healing was not detected during a period of over 2 months of standardized wound care alone. During that period of at least 2 months patients did not receive vasoac-

TABLE 2. *Baseline laboratory parameters*

Parameter	normal range	patient number 1	2	3	4	5	6	7	8	mean value (±SD)
HbA_{1c}	0.038–0.064	0.08	0.09	0.06	0.09	0.07	0.07	0.10	0.07	0.08±0.01
Hemoglobin (g/L)	140–180 g/L (f) 115–155 g/L (m)	110	152	123	122	131	134	132	148	131.50±13.75
Hematocrit	0.33–0.43 (f) 0.39–0.49 (m)	0.33	0.45	0.37	0.36	0.39	0.40	0.42	0.45	0.40±0.04
Cholesterol (mmol/L)	<6.85 mmol/L (>50y)	5.04	6.57	5.43	4.73	4.42	3.54	6.0	4.40	5.02±0.97
LDL cholesterol (mmol/L)	1.30–4.90 mmol/L	1.99	3.21	3.15	2.64	2.66	1.44	3.75	2.38	2.65±0.73
HDL-Cholesterol (mmol/L)	0.80–2.30 mmol/L (f) 0.80–1.80 mmol/L (m)	2.12	1.16	1.78	1.01	1.16	0.88	1.01	1.29	1.30±0.43
Triglycerides (mmol/L)	<1.80 mmol/L	2.04	6.47	1.08	2.39	1.28	2.66	2.72	1.57	2.53±1.71
Creatinine (μmol/L)	50–110 μmol/L	114	109	60	96	84	153	141	101	107±30

tive or rheologically active drugs or anticoagulants. Concomitant medication, antimicrobial therapy and dietary instructions remained unchanged for the length of the study. Interventional or surgical revascularization was either not technically possible or not immediately necessary; however, this was planned after improvement of wound status. All patients signed an informed consent form to participate in the study. The study protocol was approved by the local ethics committee.

Study protocol

Each patient received seven Rheopheresis treatments over a period of 11 weeks. The target for each patient for a single treatment was 100% of the patient's plasma volume. This was determined using a plasma volume nomogram adjusted for patient sex, height, weight and hematocrit (26). The first two Rheopheresis treatments were performed in week 1, followed by five treatments at 2 week intervals. Status of wound documentation, measurement of $tcPO_2$, laser Doppler flowmetry and vital capillary microscopy were performed during the baseline examination before Rheopheresis in week 0, one week after the last Rheopheresis (week 12), and at the follow-up examination 3 months after the last treatment (week 24). The duration of the study from the first baseline visit until the last examination after the 3 month follow-up time was 6 months (24 weeks).

Performance of Rheopheresis

Specialized blood and plasma therapy systems were used that automatically monitor blood and plasma flow and pressure for Rheopheresis (Hämomat Plasmomat, Diamed, Cologne, Germany). These included the OP-05 polyethylene plasma separator (Asahi Medical, Tokyo and Diamed, Cologne, Germany) and the specifically designed AR-3000 Rheofilter (effective surface of 1.7 m^2; Asahi Medical, Tokyo and Diamed, Cologne, Germany). The vascular access for the extracorporeal circuit was established by two peripheral veins. Blood was pumped at a continuous rate of 60–90 mL/min through the OP-05 plasma filter. A special plasma pump drew plasma from the outer space of plasma separator at a rate of 10–30 mL/min (typically corresponding to a third of the blood flow). Flow balance was achieved with the help of high-precision, peristaltic pumps. After plasma separation, separated plasma was pumped into the Rheofilter by the Hämomat Plasmomat (Diamed, Cologne, Germany). Molecules of molecular weight less than 900 kDa and the complete plasma fluid volume were filtered into the outer compartment of the filter and returned to the venous line of the blood circuit, where they become mixed with the separated blood cell components, finally leading back to the patient. Targeted high molecular weight plasma components were retained in the hollow fibers. Unfractionated heparin was used for anticoagulation throughout the whole study, and the system was primed with over 3 L normal saline containing unfractionated heparin (5000 IU/L) before treatment. An initial bolus (mean value 4925 ± 385 IU) was followed by continuous infusion (mean value 1354 ± 222 IU per hour). A schematic drawing of the extracorporeal circuit for Rheopheresis is shown in Fig. 1.

Wound status documentation

The primary objective of the study was to evaluate changes in wound healing of foot lesions and in wound status, according to the criteria of Wagner (Table 3) (19).

Lesion sizes and sites were recorded throughout the entire study period. Ulcerations and amputations were classified as healed status, if the wound edges were closed and there was no evidence of infection. The ulcer was classified as non-healing if there was no sign of healthy granulation tissue or wound contraction. In addition, wound status was also evaluated using the University of Texas Diabetic Wound Classification System (27).

TABLE 3. *Diabetic wound classification system for foot lesions*

Grade	Definition
0	No open lesions, complete epithelialization of pre- or postulcerative lesion.
1	Superficial lesion, limited to epidermal layers.
2	The ulcer is deeper and reaches tendon, bone or joint capsule. Bony prominence of some degree is usually present. Lesion affects subcutaneous tissue.
3	Deeper tissues are involved and there is abscess, osteomyelitis or tendinitis, usually with extension along the midfoot compartments of tendon sheaths. Lesion penetrate to tendon or bone.
4	Slight gangrene with necrosis of epidermis and subcutaneous tissue, affecting tendon and/or bone, forefoot localization.
5	Gangrene of complete foot.

Modified from Wagner (1981) and Harkless (1991). All grades, with the exception of grade 5, can be converted to a grade 0 foot. Higher stages are associated with increased risk of amputation and prolonged ulcer healing time.

Transcutaneous oximetry

Measurement of $tcPO_2$ is a non-invasive method for investigation of local arterial blood flow and skin oxygenation. Values of $tcPO_2$ were determined to assess the severity and clinical progression of peripheral arterial occlusive disease and to evaluate cutaneous ischemia, particularly in advanced stages of atherosclerosis of the lower limbs.

In the investigation presented here, measurements of lower extremity $tcPO_2$ (expressed in millimeters of mercury [mm Hg]) were performed repeatedly at 37°C and 44°C under uniform standard conditions using the $TcPO_2$ Monitor (Medizinelektronik Fred Lawrenz, Bad Soden, Germany) connected to a chart recorder. At 44°C, the O_2 partial pressure of the skin surface is independent of the blood pressure, being solely dependent on the arterial O_2 partial pressure of the blood (28).

After calibration of the sensor, periwound $tcPO_2$ values were measured immediately close to the greatest wound lesion. Likewise, the $tcPO_2$ of the contralateral dorsum of the foot was measured. The oxygen sensor, a modified Clark electrode, was fixed to the skin surface with a double-sided adhesive ring including an NaCl 0.9% solution. The electrodes quantify oxygen content by measuring the rate of oxygen reduction at the cathode. The cathode and anode were suspended in an electrolyte solution behind an oxygen-permeable Teflon membrane. A constant voltage was applied between the electrodes and the current voltage was measured (6).

Laser Doppler flowmetry

Laser Doppler flowmetry is a non-invasive, selective measurement method for skin blood flow. It is based on the intensity of laser light scattered by erythrocytes moving under the sampling probe. Laser Doppler flowmetry is mainly a marker for the status of the skin circulation. Skin perfusion must be assessed under constant temperature conditions and after an acclimatization time of 20 min. The laser Doppler signal is converted into flux and is expressed in arbitrary units (AU). This flux is mainly the product of red blood cell concentration and speed. Measurements fluctuate both inter- and intraindividually. The standard segmental laser Doppler technique was employed to measure skin perfusion near the ulcer and the dorsalis pedis of the damaged foot. Laser Doppler tracings were evaluated according to previously established criteria (29) using the laser blood flow monitor *MBF3D* (Moor Instruments Axminster, UK; Lawrenz Medizin-Elektronik Sulzbach/Taunus, Germany).

Vital capillary microscopy

Capillaries change both morphologically and dynamically in patients with reduced arterial circulation. Capillaroscopy of the skin was used to directly determine the quantity and quality of the skin's nutritive capillaries. Dynamic videocapillaroscopy was the technique used to measure, above all, the capillary blood cell velocity (CBV, expressed in millimeters per second [mm/s]). The CBV was measured near the wound lesion and at the toe of the contralateral foot. Observations were recorded with a specialized microscopic television system (AG-7350, Panasonic, Germany) using a computerized video-photometric system (*Capimage*, Fa. Zeintl Medical Software Engineering, Heidelberg, Germany). The CBV evaluation is conducted with the 'Line Shift Diagram'. Capillaries were visualized using the *CapiScope microScopeman MS-500* (Lawrenz Medizin-Elektonik, Sulzbach/Taunus, Germany) and a microscopic television system (*AG-7350*, Panasonic, Germany). Video recordings were made to count the number of capillaries per mm^2 near the wound lesion. The velocity data of both feet over a period of at least 1 min were registered, and the average values of at least four capillaries per sequence were determined.

Blood sampling and laboratory parameters

Blood samples were collected immediately before and after each Rheopheresis session. Laboratory parameters such as blood count, creatinine, HbA_{1c}, LDL cholesterol, triglycerides, fibrinogen, immunoglobulin M (IgM), a_2-macroglobulin, fibronectin, vWF and total protein, were measured by routinely applied clinical chemistry procedures using commercial kits. Plasma viscosity was measured with a capillary tube plasma viscosimeter (Fresenius, Germany). Concentrations of TM, ICAM-1, VCAM-1 and E-selectin were measured before each Rheopheresis session using commercially available kits of enzyme-linked immunosorbent assays (ELISA) (American Diagnostica Inc., Greenwich, UK; MedSystems Diagnostics GmbH, Vienna, Austria). The latex immunonephelometry method was employed to measure hs-CRP before each Rheopheresis session; this uses a high-sensitivity assay system for CRP with a lower limit of sensitivity of 0.03 mg/L (Tina-quant hs-CRP, Roche, Germany). vWF-antigen (vWF-Ag) was determined with an STA clot analyzer (STA Liatest vWF, Roche, Diagnostica Stago, Germany).

Descriptive statistical analysis

All values of normally distributed variables are presented as mean with standard deviations (± SD) The statistic program *Statistical Package for Social*

TABLE 4. *Changes in wound healing as assessed using the diabetic wound classification system of Wagner*

	improved				unchanged		deteriorated	
Patient Number	1	2	4	6	5	8	3	7
WS Baseline (week 0)	2	2	2	2	2	2	5	4
WS Final (week 12)	0	1	0	1	2	2	5	5
WS Follow-up (week 24)	0	1	0	1	not done	2	not done	not done
Amputation	no	no	no	no	yes	yes	yes	yes
Level of amputation					minor	minor	major	major

WS, Wagner Stage.

Science (SPSS 10.0) was used for data analysis. This allows descriptive statistics and includes the Wilcoxon test for matched pairs.

RESULTS

Safety of Rheopheresis treatments

In accordance with the study protocol, eight patients were recruited for this pilot trial and their progress was followed over a time period of 6 months. In total, 53 Rheopheresis treatments with a mean treated plasma volume of 3016 ± 518 mL were performed. Seven patients received seven Rheopheresis treatments; the treatment series was not completed in one patient because a major lower extremity amputation had to be performed after the fourth session. No substitution of fresh frozen plasma, albumin or any other plasma products was necessary. There were no occurrences of clinically relevant adverse events which necessitated an interruption of Rheopheresis treatment. Rheopheresis did not have any severe side-effects on hemodynamic parameters, such as blood pressure and heart rate. Transient hypotension occurred in four out of 53 treatments (7.5%), and could be easily controlled. No episodes of severe arterial hypotension requiring the application of vasoactive drugs were observed. Safety parameters (Table 2) showed no clinically relevant changes during the treatment period. Hemoglobin and creatinine were approximately within normal ranges, whereas HbA_{1c} was slightly elevated. Metabolic control, assessed by blood glucose levels and HbA_{1c}, remained stable after each treatment session. A mild decrease of HbA_{1c} was observed throughout the whole study period (mean HbA_{1c} at baseline was 0.08 ± 0.01, and was 0.07 ± 0.01 at final visit).

Wound healing and transcutaneous oximetry

The healing of lesions was assessed using the criteria of Wagner (Table 4). Improvements with respect to baseline wound status were observed in four patients, and were correlated with the mean $tcPO_2$ values measured after the treatment period. Changes in $tcPO_2$ from the baseline examination to week 12, from week 12 to the follow-up examination in week 24, or from the baseline examination to week 24 were expressed as delta $tcPO_2$ (Δ $tcPO_2$) (Table 5). Improvements in wound healing in three patients (patients 1, 2 and 4) which occurred during the study period are illustrated in Fig. 2.

In four out of eight patients (patients 1, 2, 4 and 6) the treatment series resulted in accelerated wound healing of foot ulcers, with the improvement in Wagner grade being associated with a pronounced increase in $tcPO_2$. After the last treatment session in week 12, the average Δ $tcPO_2$ was 13.23 ±

TABLE 5. *Results of $tcPO_2$ measurements in patients with improved, unchanged and deteriorated wound healing*

	improved				unchanged		deteriorated	
Patient number	1	2	4	6	5	8	3	7
$tcPO_2$ (mm Hg)								
Baseline (week 0)	1.1	9.2	4.1	19	3.8	9.1	9	1.2
Final (week 12)	21.5	17.2	26.2	21.4	23.3	20	2	3.6
Follow-up (week 24)	19.4	26.2	17.6	23	no value	24	no value	no value
Δ $tcPO_2$ (mm Hg)								
Δ1 (week 0–12)	20.4	8	22.1	2.4	19.5	10.9	−7	2.4
Δ2 (week 12–24)	−2.1	9	−8.6	1.6	no value	4	no value	no value
Δ3 (week 0–24)	18.3	17	13.5	4	no value	14.9	no value	no value
mean Δ $tcPO_2$ (mm Hg)								
mean Δ1 (week 0–12)		13.23 ± 9.57			15.20 ± 6.08		−2.30 ± 6.65	

$P < 0.05$, Wilcoxon test for matched pairs; Values are given as mean ± S.D.

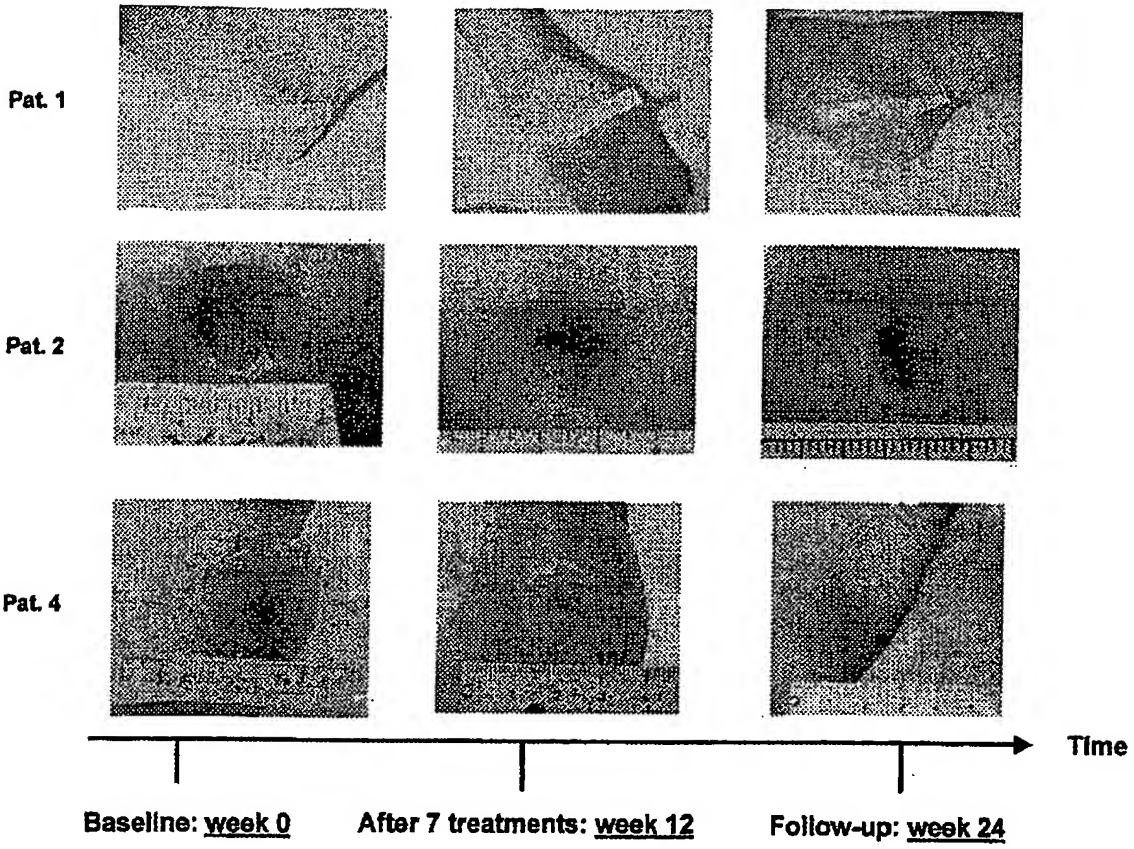

FIG. 2. Diabetic foot ulcers with improvement of wound healing as assessed by the Wagner criteria are shown.

9.57 mm Hg ($P < 0.05$, Wilcoxon test for matched pairs) and remained stable until the follow-up examination in week 24 (Table 5) (Fig. 3a).

In two out of eight patients (patients 5 and 8) who had Wagner stage 2 grades at the baseline examination, wound healing, as assessed by Wagner criteria, did not change. Mean Δ $tcPO_2$ was 15.20 ± 6.08 mm Hg ($P < 0.05$, Wilcoxon test for matched pairs) within the first 12 weeks, and remained stable in the follow-up interval. Due to the initial low $tcPO_2$ levels in both patients, performance of minor amputations bore the risk of non-healing ulcers and subsequent infection. $tcPO_2$ values improved after Rheopheresis, and minor amputations were successfully performed, followed by healing of lesions in both patients. Surgical revascularization could be postponed (Table 5) (Fig. 3b).

In two out of eight patients (patients 3 and 7) with Wagner 4 and 5 lesions at study entry, $tcPO_2$ and foot lesions did not profit from the treatment series. Mean Δ $tcPO_2$ was -2.3 ± 6.65 mm Hg ($P < 0.05$, Wilcoxon test for matched pairs) and amputations at a proximal level of the extremity could not be delayed. In one of these patients, gangrene of the complete forefoot developed with subsequent bacterial infection. Rheopheresis did not result in an improvement of wound status and major amputation had to be performed after the fourth treatment (Table 5) (Fig. 3c).

Pronounced increases in $tcPO_2$ were observed in the six patients who showed improved or unchanged wound healing according to Wagner criteria; this supports the idea that $tcPO_2$ is the parameter of highest clinical relevance. In these six patients (patients 1, 2, 4, 5, 6, 8), mean Δ $tcPO_2$ was 13.88 ± 7.96 mm Hg ($P < 0.05$, Wilcoxon test for matched pairs) within the first 12 weeks of the treatment period.

In addition, wounds were also graded according to the University of Texas Diabetic Wound Classification System (27). With respect to the clinical course of wound healing, the results of this wound classification system were essentially identical to those

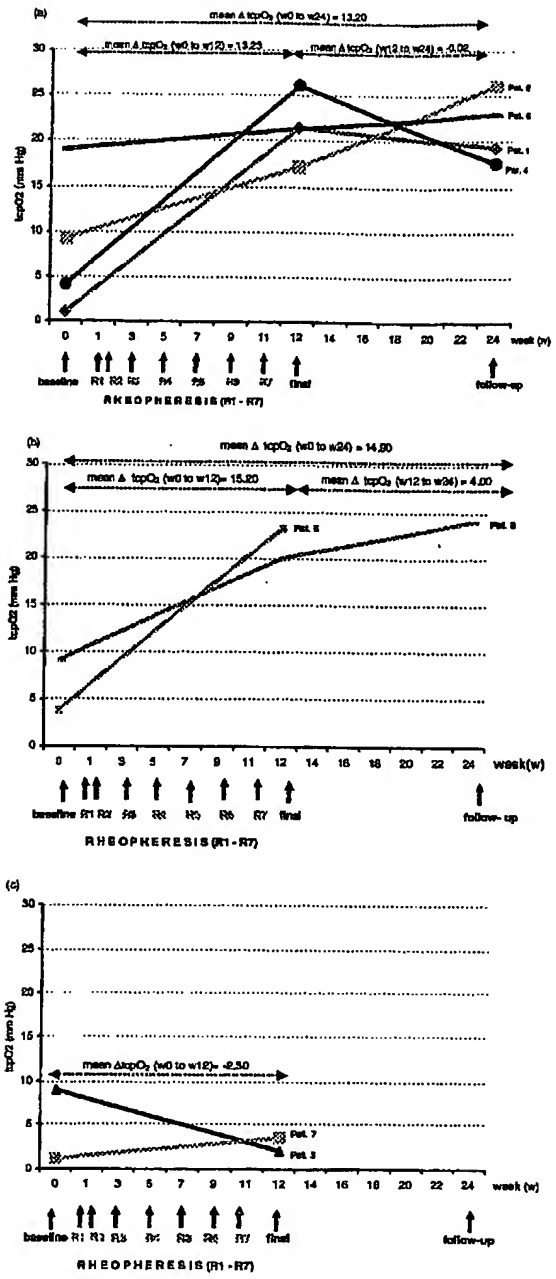

FIG. 3. (a) Change in $tcPO_2$ in four patients with *improvement* of wound healing as assessed by the Wagner criteria. (b) Change in $tcPO_2$ in two patients with *no change* of wound healing as assessed by the Wagner criteria. (c) Change in $tcPO_2$ in two patients with *deterioration* of wound healing as assessed by the Wagner criteria.

from the Wagner assessment method; they did not yield a better correlation between the clinical course of wound healing and the treatment effect of Rheopheresis. Results are therefore not presented in detail.

Laser Doppler flowmetry and vital capillary microscopy

Laser Doppler flowmetry was used to quantify the skin blood flow. Flux signals were recorded, registered and digitalized for off-line evaluation. In this investigation, results of laser Doppler flowmetry and vital capillary microscopy were evaluated, but measured values did not correlate with the clinical course of wound healing. Furthermore, no association between $tcPO_2$ values and laser Doppler flowmetry or capillary microscopy was observed.

Hemorheological laboratory paramters

Rheopheresis resulted in a decrease in the concentration of hemorheologically relevant plasma proteins, such as LDL cholesterol, fibrinogen, IgM, α_2-macroglobulin, fibronectin, and vWF. Mean changes in laboratory parameters before and after Rheopheresis are given in Table 6.

There is a linear relationship between plasma viscosity and levels of certain rheologically active macromolecules, especially large proteins such as LDL cholesterol, fibrinogen, vWF, fibronectin, IgM and α_2-macroglobulin (12,30). In this investigation, plasma viscosity was effectively diminished, associated with the reduction of LDL cholesterol, fibrinogen, and concentrations of IgM and α_2-macroglobulin. In addition, fibronectin, vWF and total protein levels were reduced by $43.05 \pm 9.74\%$, $55.15 \pm 5.69\%$. and $20.70 \pm 4.53\%$, respectively, immediately after the treatment session. Mean pretreatment values of total protein (65.30 ± 3.19 g/L) and IgM (0.97 ± 0.50 g/L) were not affected by Rheopheresis, and remained stable during the study period of 6 months. Pretreatment values reflect redistribution of plasmaproteins from extravascular spaces as well as continuous biosynthesis during the interval between Rheopheresis treatments.

Molecular markers of endothelial function and inflammatory state

During the treatment period, fibronectin, vWF and hs-CRP were measured and found to be within approximately normal ranges in patients with improved wound healing. Figures 4,5 and 6 show that levels of these solutes decreased during the period of seven Rheopheresis treatments in patients with improved clinical outcome. As opposed to this,

TABLE 6. *Influence of Rheopheresis on plasma viscosity, LDL cholesterol, fibrinogen, immunoglobulin M (Ig M), α-macroglobulin, fibronectin, von Willebrand factor (vWF) and total protein (mean pre- and post-treatment levels and mean percentage reduction; eight patients, 53 Rheopheresis sessions, mean treated plasma volume: 3016 ± 518 mL)*

Parameter	Pre-treatment	Post-treatment	Mean percentage reduction
Plasma viscosity (mPas)	1.22 ± 0.10	1.02 ± 0.11	15.81 ± 8.08
LDL cholesterol (mmol/L)	2.50 ± 0.43	1.15 ± 0.46	54.65 ± 11.99
Fibrinogen (g/L)	4.28 ± 0.92	2.03 ± 0.76	53.23 ± 9.56
Ig M (g/L)	0.97 ± 0.50	0.47 ± 0.24	48.39 ± 12.44
α_2-macroglobulin (g/L)	1.89 ± 0.57	0.91 ± 0.35	52.41 ± 8.33
Fibronectin (g/L)	0.48 ± 0.06	0.27 ± 0.05	43.05 ± 9.74
vWF (g/L)	2.18 ± 0.39	0.93 ± 0.37	55.15 ± 5.69
Total Protein (g/L)	65.30 ± 3.19	51.91 ± 5.09	20.70 ± 4.53

Values are given as mean ± SD; Plasma concentrations were measured before (pretreatment) and after (post-treatment) Rheopheresis.

fibronectin, vWF and hs-CRP values tended to increase in those patients exhibiting a deterioration of wound healing, however, they did not reach statistical significance due to the low patient number.

The mean pretreatment value of vWF was 1.97 ± 0.25 in patients with improved clinical outcome (patients 1, 2, 4, 5, 6 and 8), and 2.65 ± 0.08 in patients exhibiting a deterioration of wound healing (patients 3 and 7). The mean hs-CRP pretreatment level was 4.57 ± 2.81 mg/L in patients with improved wound healing, and 33.65 ± 26.02 mg/L in patients with a deterioration of wound status.

In this investigation, no significant correlation between markers of endothelial cell dysfunction (e.g. TM, ICAM-1, VCAM-1 and E-selectin) and clinical outcome could be observed, although all of these parameters were reduced immediately after each treatment session.

DISCUSSION

In this pilot trial, eight patients with ischemic diabetic foot syndrome and long-lasting, non-healing foot lesions were investigated. Rheopheresis is a safe and effective mode of therapeutic apheresis for the treatment of microcirculatory disorders (11,16). The effect of Rheopheresis on wound healing was analyzed using the wound classification system of Wagner. Tissue repair was evaluated by measuring values of $tcPO_2$, which correlate with microcirculatory impairment in patients with diabetic foot, even at early stages (6). Hemorheological properties were characterized by laboratory parameters. The results demonstrated that both wound healing of diabetic foot ulcers (as assessed by the Wagner criteria) and the corresponding $tcPO_2$ values could be improved by Rheopheresis in four out of eight patients. A general increase in oxygen supply of peripheral tissues around the gangrene lesions was also observed in association with improvements of Wagner criteria. Analysis of $tcPO_2$ levels revealed a tendency towards lower $tcPO_2$ levels in patients who subsequently underwent major amputation, whereas $tcPO_2$ values were higher in those limbs with improved wound healing. Increases in $tcPO_2$ were maintained even three months after the last Rheopheresis treatment. Trials investigating hyperbaric oxygen therapy in

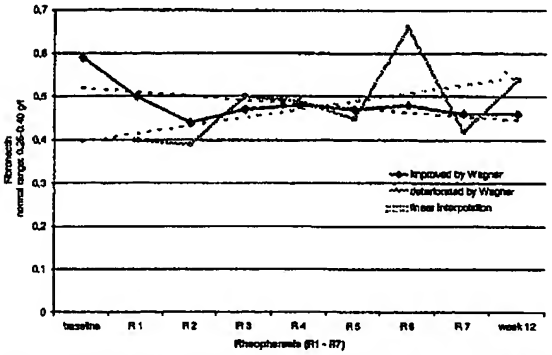

FIG. 4. Pre-treatment mean values of fibronectin in eight patients with ischemic diabetic foot syndrome treated with Rheopheresis (pretreatment, mean values).

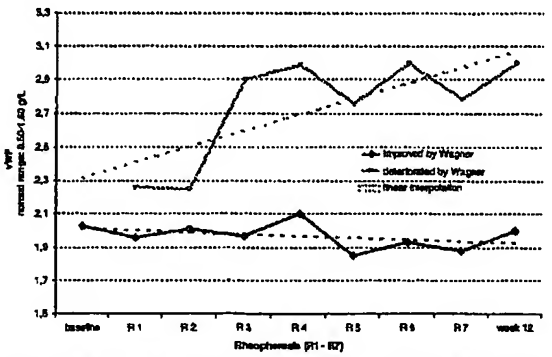

FIG. 5. Pre-treatment mean values of von Willebrand factor (vWF) in eight patients with ischemic diabetic foot syndrome treated with Rheopheresis.

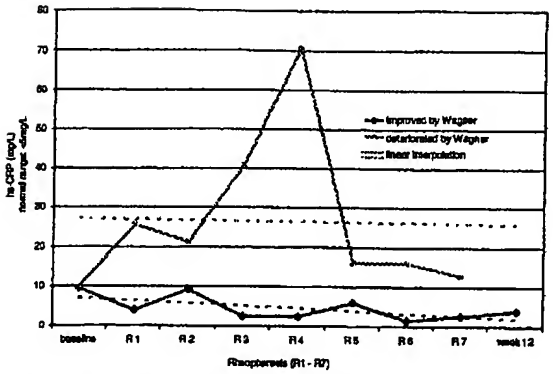

FIG. 6. Pre-treatment mean values of high-sensitivity C-reactive protein (hs-CRP) in eight patients with ischemic diabetic foot syndrome treated with Rheopheresis (pretreatment, mean values).

patients with diabetic foot ulcers have reported successful results after 40–60 single treatments; however, $tcPO_2$ levels remained above baseline levels only for 3–4 h after each of these treatments (31,32). It is important for the understanding of the therapeutic potential of Rheopheresis, that the repetitive single pulses of plasma protein elimination with associated reduction of plasma viscosity can result in sustained improvements of microcirculation. This of course is an hypothesis, but it is confirmed by available clinical data for age-related macular degeneration (17). Rheopheresis directly targets risk factors and pathophysiologically relevant factors of the progression of diabetic angiopathy lowering plasma viscosity, and eliminating fibrinogen, cholesterol, vWF, and α^2-macroglobulin. A functional reserve might exist in the blood vessels with its surrounding tissue affected by diabetic microangiopathy, which is determined by the individual pattern of reversible and irreversible morphologic changes. Diabetic foot syndrome spontaneously has a chronic progressive course. Irreversible functional and morphologic changes increase over time. The capacity of the individual functional reserve cannot be assessed by any diagnostic procedure. The hypothetic goal of the Rheopheresis treatment is to restore and activate or stabilize the functional reserve. In general, the regenerative potential is highly dependent upon microenvironmental conditions, i.e. the degree of morphologic changes and the microcirculatory impairment at cellular and molecular levels, e.g. decreased oxygen supply. Rheopheresis treatment resulted in sustained improvement of tissue oxygenation induced by the repeated therapy pulses. Unfortunately, there is currently no experimental model system to test that hypothesis.

Although an association between hemorheological parameters, laser Doppler flowmetry and capillary perfusion was reported, the results of laser Doppler flowmetry and vital capillary microscopy did not reflect the clinical course to an acceptable extent (33): a significant correlation was not found in this investigation between these measurements and the course of wound healing, confirming that the value of these microcirculatory diagnostic techniques is limited to the monitoring of individual cases (22). Pathological patterns of laser Doppler flowmetry were reported in larger groups of patients with ischemic diabetic foot syndrome, and a decreased capillary density with less visible capillaries has been implicated as a risk factor for consecutive amputation and delayed wound healing (22).

LDL cholesterol, fibrinogen, α_2-macroglobulin, fibronectin and vWF were effectively removed by Rheopheresis. The reduction in fibrinogen of 53.23% and in LDL cholesterol of 54.65% resulted in improved hemorheological properties, as indicated by a decrease in plasma viscosity of 15.81%. The fundamental therapeutic basis of Rheopheresis is the reduction of blood and plasma viscosity: this results in improvements in microcirculation and blood flow, and in an enhancement in local oxygenation of gangrenous tissue.

The degree of metabolic control, the presence of ischemia and infection, intensive wound care, and good patient education are factors that affect development and healing of diabetic foot ulcers, as well as the number of major amputations. Skin capillary ischemia seemed to be more pronounced in patients with bad metabolic control, defined as $HbA_{1c} > 0.07$ (normal range: 0.038–0.064), showing an association between significantly increased HbA_{1c} values and the development of late diabetic complications (6). Patients investigated in this pilot trial failed to improve under such an optimized regimen before commencement of Rheopheresis treatment.

Published reports on endothelial cell adhesion molecules in diabetic patients are contradictory. Circulating levels of proinflammatory cytokines and adhesion molecules are elevated in patients with diabetes mellitus, linking endothelial dysfunction, atherogenesis and thrombogenesis to foot ulceration, and suggesting a role for systemic inflammation in diabetogenesis (23). Inflammatory activity can promote vascular injury by modifying lipoprotein structure and function, changing the composition of plasma proteins and causing alterations in the vascular endothelium (34,35). No clear correlation between the measured endothelial-specific parameters and the extent of wound healing was found in

this investigation. However, levels of fibronectin, vWF and hs-CRP in two patients with major amputations who did not profit from the treatment were elevated and increased further during the treatment period, while these levels tended to decrease in the patients with improved wound status, values being within approximately normal ranges. Increased expression of fibronectin, an extracellular matrix glycoprotein, is found in tissues involved in diabetic complications (36). Fibronectin is typically found in thickened basement membranes, such as are characteristic of capillaries and small vessels of diabetic tissue. Plasma fibronectin was shown to be a marker of endothelial cell dysfunction in diabetes, and was associated with the occurrence and progression of microangiopathy (37). The pathogenic mechanism may be a relationship between fibronectin and blood viscosity (38): an accumulation of fibronectin in tissue and a corresponding increase in plasma viscosity may play a role in the progression of diabetic microangiopathy.

Rheopheresis appears to be a suitable adjunct therapy for diseases involving severe disturbance of microcirculation, especially when previous therapeutic options were not sufficiently effective and invasive procedures cannot be applied. Values of $tcPO_2$ were still above baseline levels at the 3 month follow-up examination, suggesting a sustained, beneficial effect on tissue oxygenation, which is closely related to tissue function. This observation parallels results in patients with age-related macular degeneration, a disease with progressive deterioration of retinal function: a sustained treatment effect could be demonstrated 12 months after the initial series of 8–10 treatments for this (16,17). Potential candidates for Rheopheresis treatment include those diabetic patients with wound lesions of Wagner stage 2–3 who do not respond to standard treatment (such as surgical revascularization) and where amputation appears advisable. The achievement of clinically relevant wound healing in four out of eight patients is encouraging.

Recently, the results of a pilot study using a fibrinogen adsorber to decrease fibrinogen levels over a period of 28 days by repetitive treatments every 2–3 days were published (39). A mean pretreatment decrease in fibrinogen levels of 45% was observed in 10 patients with diabetic foot ulcers after 6–26 treatments. Improvements in wound healing were observed in eight out of ten patients. In one patient, improvement of wound healing was observed in addition to successful minor amputation, and major amputation had to be performed after the sixth treatment in another patient.

A final conclusion cannot be drawn in the present study due to the low number of patients enrolled. The promising potential of Rheopheresis as an adjunct therapeutic option for patients with diabetic foot ulcers and in the long-term prevention of major amputation in diabetic patients must be further investigated in future clinical trials.

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